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(54) Hair growth stimulation with nitroxide and other radicals.

(57) Hair growth stimulation with nitroxide and other radicals. A nitroxide radical source compound is applied topically with an adjuvant selected from reducing agents, hydroxyl radical scavengers and antioxidants to activate formation of the nitroxide radical and/or to protect the nitroxide radical from reaction with other free radicals.

Also disclosed is a kit for preparing a unit dose for topical application of a nitric oxide generating compound and a reducing agent reactive therewith to form nitric oxide on or in the skin. Other adjuvants include SOD and antiandrogens. Various other radical-forming hair growth stimulants and preparations thereof are disclosed.

EP 0 327 263 A1

HAIR GROWTH STIMULATION WITH NITROXIDE AND OTHER RADICALS

This invention relates to a composition and method for stimulating hair growth, and to a kit for preparation of such a composition.

Various treatments have been available for stimulating the cosmetic growth of hair, and for conditions such as male and female pattern baldness and alopecia areata. Several substances were known to be effective when administered internally, but had undesirable concomitant systemic effects and the hypertrichosis was not confined to the scalp area. In an effort to avoid these side effects and to confine the hypertrichosis to the scalp area, several attempts were made to apply such substances in a topical preparation to the affected area. However, such attempts had generally been only marginally successful, and the results obtained with the topical preparation containing the orally effective substances were comparable to and generally little better than those obtained with topical application of the carrier only.

U.S. Patent 2,986,573 described a process for treating hypertension by administering a 1,2,4-benzothiadiazine 1,1-dioxide, otherwise unsubstituted in the heterocyclic portion of the nucleus, having a saturated lower aliphatic hydrocarbon radical in the 3-position and a chlorine atom or its equivalent on the benzenoid portion of the nucleus in the 6- or 7-position.

U.S. Patent 4,184,039 described the development of uncontrolled hair growth in patients treated orally with 1,2,4-benzothiadiazine 1,1-dioxides; and also described topical application of 6-chloro-3-dimethylaminoethoxymethyl-2H-1,2,4-benzothiadiazine 1,1-dioxide and 6-chloro-3-cyclohexenyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide in DMSO and in suspension to promote hair growth.

U.S. Patents 4,139,619 and 4,596,812 described a process for stimulating the growth of mammalian hair by the application of 6-amino-4-(substituted amino)-1,2-dihydro-1-hydroxy-2-iminopyrimidines to mammalian skin in association with a topical pharmaceutical carrier.

U.S. Patent 4,347,245 described a composition containing spironolactone in a liquid carrier such as alcohol, urea, mineral oil or white petrolatum.

Stewart, M.E. et al., "Antiandrogens and the Skin," International Journal of Dermatology, Vol. 17, pp. 167-179 (1978) described the application to the foreheads of acne patients of 10% cyproterone in 50% aqueous dimethyl sulfoxide, with no reduction in sebum secretion or improvement in acne being produced.

U.S. Patent 4,367,227 described a composition for reducing sebum secretion when applied to the skin, which composition contained cyproterone acetate dissolved in a C_2-C_3 aliphatic alcohol.

The present invention relates to hair growth stimulation, for example, in individuals with pattern hair loss. It has been discovered that certain free radical species can stimulate hair growth particularly when repeatedly applied in a topical preparation to the site at which stimulated hair growth is desired.

In one aspect, the invention provides a method for stimulating hair growth which includes topically applying to skin a pharmacologically acceptable stable free radical source compound, and concurrently therewith, a pharmacologically acceptable adjuvant which enhances the activity of or synergizes with the free radical-forming compound to stimulate hair growth.

Stable free radical-forming compounds include, for example, certain compounds having nitroxide or sulfoxide moieties which form relatively stable nitroxide and sulfoxide radicals, compounds which generate nitric oxide in the presence of a reducing agent, and compounds which generate oxygen radicals such as peroxides. Adjuvants include, for example, hydroxyl radical scavengers reducing agents and antioxidants which may either induce formation of free radicals from the free radical-forming compounds, or stabilize the free radicals formed therefrom by limiting reaction with other reactive radicals. The topical application can also include a combination of such adjuvants, such as, for example, a reducing agent and superoxide dismutase activity. Optically, the adjuvant may also include an antiandrogen.

In one embodiment, this method involves the repeated topical application of a preparation containing a compound forming a stable free radical and the adjuvant. A mixture of the free radical source and the adjuvant are relatively unreactive, and can be premixed and stored until application. In another embodiment, a compound capable of generating free radicals is mixed with a reducing agent in an amount effective to form the free radicals at or near the time of topical application. For example, a first topical preparation containing an organic nitrate or an alkali metal nitrite can be mixed with a reducing agent in a second topical preparation and topically applied so that resulting nitric oxide radicals are generated in situ at the site of application.

In another aspect, the invention provides a kit for preparing a unit dosage of a hair growth stimulating preparation. The kit includes a unit dosage amount of a pharmacologically acceptable nitroxide radical-forming compound in a pharmaceutical carrier, a unit dosage amount of a pharmacologically acceptable reducing agent in a pharmaceutical carrier, the reducing agent being reactive with the radical-forming

compound to form nitroxide radicals, means for separating the dosage amounts from each other prior to dispensing, and means for dispensing the unit dosage amounts. The dispensing means may also include means for mixing the unit dosage amounts. The kit can be used to prepare a topical preparation which has been activated to generate nitric oxide radicals at the site of application to stimulate hair growth following repeated applications.

In still another aspect, the invention provides a topical preparation for stimulating hair growth from skin which includes, in association with a topical pharmaceutical carrier, a pharmacologically acceptable stable radical-forming compound and a pharmacologically acceptable adjuvant. The adjuvant comprises a hydroxyl radical scavenger, a reducing agent, an antioxidant and/or an antiandrogen.

The method of the present invention involves the concurrent topical application of a stable free radical-forming compound as a hair growth stimulant and adjuvant which either activates the free radical formation and/or protects the stable free radicals once formed from reaction with other reactive species such as hydroxyl radicals, for example. The hair growth stimulant and adjuvant are usually repeatedly applied to the scalp or other site at which hair growth is desired. The application is typically made once or twice daily, although a greater or lesser frequency of application can be used, if desired. Generally, the frequency of application is greater during the first three to six or twelve months of treatment, e.g. twice a day, but the application frequency usually can be reduced to once every two to three days as a maintenance program after the desired hair growth stimulation has been achieved.

The carrier or carriers in which the free radical-forming compound, adjuvant and any other active ingredients will generally be substantially homogenously dispersed, e.g. at 10-100 mM, is preferably an occlusive or semi-occlusive preparation which may be a water-in-oil emulsion, but is most preferably an oil-in-water emulsion. As used herein, the terms "occlusive" or "semi-occlusive" are used in reference to a carrier which substantially prevents or inhibits, respectively, evaporation of water from the skin to which it is applied. As examples of non-occlusive carriers, there may be mentioned water, urea, alcohols and glycols such as methanol, ethanol, propanol, butanol, ethylene glycol and propylene glycol, and the like. Suitable water-in-oil emulsions are commercially available under the designations Aquaphor, cold cream, Eucerin, hydrous lanolin, Hydrosorb, hydrophilic petrolatum, Nivea, Polysorb, Qualatum and Velvachol. Suitable oil-in-water emulsions are available commercially under the designations acid mantle cream, Almay emulsion cream, Cetaphil, Dermabase, Dermovan, hydrophilic ointment, Keri cream, Lubriderm cream, Multibase cream, Neobase cream, Univase cream, Vanibase cream, and Wibi. The carrier may further obtain various other emollients, emulsifiers, water, perfumes, colorants, preservatives and the like. In a preferred embodiment, the carrier comprises the Dermovan emulsion, propylene glycol and water.

According to the method of the invention, the free radical source and adjuvant are concurrently applied topically to the skin to be treated, such as the scalp. Preferably, the application is once a day with a sufficient amount of the free radical source and adjuvant to cover the area at which the stimulation of hair growth is desired. Generally, results are improved when the active ingredients are applied after water-soaking the skin. Thus, a preferred embodiment of the method is convenient in that the free radical source and adjuvant can be applied once daily immediately following bathing. However, it is desirable to leave the medication in place for a period of time and it should not be washed off for at least several hours, probably at least eight hours. Generally, best results are obtained in treatment of balding or thinly-haired scalp areas in which hair loss has not occurred for a period of time substantially in excess of about three to five years. The effectiveness also depends, although to a lesser degree, inversely on the age of the user. As used herein, the term "hair growth stimulation" generally includes conversion of vellus hairs to terminal hairs, an increase in shaft diameter, an increase in the rate of growth of terminal and vellus hairs, and possibly follicular neogenesis.

In one typical embodiment a premixed preparation of minoxidil and DMSO is applied to the scalp in a pharmaceutical carrier for three to six months to stimulate hair growth. In another typical embodiment, sodium nitrite is applied to the scalp, followed by a separate application of a reducing agent such as cysteine.

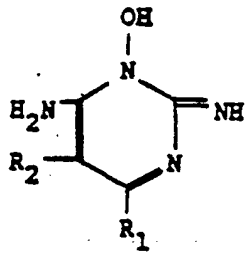
By concurrent application, it is meant that the free radical source and adjuvant are applied together or at or near the same time so that the adjuvant functions with the free radical source to enhance the hair growth stimulation relative to that which would be obtained by application of the free radical source or the adjuvant alone. Generally, the adjuvant will be active in mixture, or in the skin to which it is applied, with the free radical source to form free radicals by reaction therewith, or otherwise by shifting equilibrium between the excited state or free radical form of the free radical source toward a greater preponderance of the excited state species. Alternatively, the adjuvant may be active in the presence of the free radicals formed from the free radical source compound to protect the same by reacting with other undesired free radicals, or inhibiting formation thereof, which undesired free radicals might otherwise react with or inactivate the

desired free radicals. In this manner the free radicals formed from the free radical source are available in the skin to stimulate hair growth. For example, the free radical source compound and adjuvant may be premixed in the same carrier, mixed at or near the time of their application to the skin, or applied separately within a period of time sufficient for concurrent activity, e.g. within several minutes to one hour or more of each other.

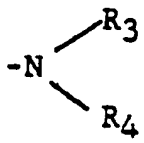
Free radicals are molecules having one or more electrons with unpaired spins and are generally short-lived, reactive or unstable chemical species, conventionally having a half life of the order of less than about 10 msec. However, relatively stable free radicals having a half life much longer than this can be formed in some species due to steric protection, resonance stabilization and other means of protecting the unpaired electron. For example, nitric oxide radicals have a half-life of the order of 30 seconds in biological systems, while spin labels and traps are, or may form, free radicals which are nearly indefinitely stable. The formation of stable free radicals in a substance (other than a spin label) is generally attributable to electron acceptance or donation from other radicals or reducing and/or oxidizing species, and is generally confirmed by electron spin resonance spectrometry.

Stable free radical-forming compounds generally include resonance stabilized nitroxy compounds, sulfoxy compounds, other nitrogenous compounds and the like. A preferred class of compounds include the nitroxide radical-forming compounds such as nitrovasodilators and spin traps and labels. As exemplary nitrovasodialtors which may form relatively stable nitroxide radical species there may be mentioned, nitroglycerin, amyl nitrate, sodium nitroprusside, sodium nitrite, and the like.

Particularly preferrred nitrovasodilators are minoxidil and the related pyrimidine N-oxides described in U.S. Patent 3,461,461 which is incorporated herein by reference. Minoxidil is 6-amino-1,2-dihydro-1-hydroxy-2-imino-4-piperidinopyrimidine and also includes tautomers such as 2,4-diamino-6-piperidinopyrimidine 3-oxide. Also contemplated are the other free base forms and acid addition salts of the 6-amino-4-(substituted amino)-1,2-dihydro-1-hydroxy-2-iminopyrimidines described in U.S. Patent 3,461,461. Briefly, such compounds include 1,2-dihydro-1-hydroxypyrimidines of the formula:



wherein R_1 is a moiety selected from moieties of the formula:



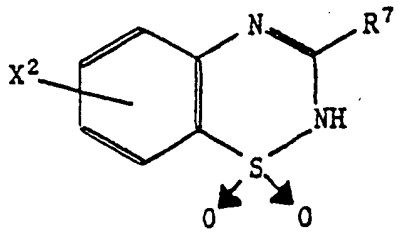
wherein R_3 and R_4 are selected from hydrogen, lower alkyl, lower alkenyl, lower aralkyl, and lower cycloalkyl, and taken together, R_3 and R_4 may be a heterocyclic moiety selected from aziridinyl, azetidinyl, pyrrolidinyl, piperidino, hexahydroazepinyl, heptamethylenimino, octamethylenimino, morpholino and 4-lower-alkyl-piperazinyl, each of the hetrocyclic moieties having attached as substituents on the carbon atom thereof 0-3 lower alkyl groups, hydroxy or alkoxy, and wherein R_2 is selected from hydrogen, lower alkyl, lower alkenyl, lower alkoxyalkyl, lower cycloalkyl, lower aryl, lower aralkyl, lower alkaryl, lower alkoxyaralkyl, and lower haloaralkyl and the tautomers and pharmacologically acceptable acid addition salts thereof. Particularly preferred is minoxidil sulfate. These compounds and their preparation are also described in U.S. Patents 3,382,247 and 3,644,364.

Other stable nitroxide radical-forming compounds preferred as hair growth stimulants in the present invention include nitroxide spin labels and spin traps. Exemplary of these are: melanin; 4,4-dimethyl-3-oxazolinyloxy (hereinafter "doxyl") and derivatives such as 3-doxyl-5α-cholestane, 3-doxyl-17β-hydroxy-5α-androstane, 5-doxylstearic acid, 7-doxylstearic acid, 12-doxylstearic acid, 16-doxylstearic acid, 5-doxyl-stearic acid methyl ester, 7-doxylstearic acid methyl ester, 12-doxylstearic acid methyl ester, 16-doxyl-

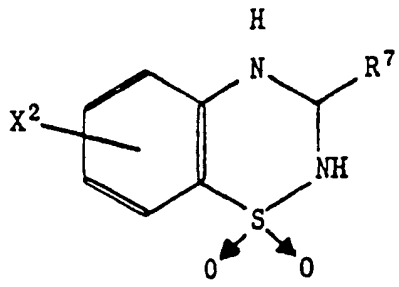
stearic acid methyl ester and the like; 2,2,5,5-tetramethyl-1-pyrrolidinyloxyl (hereinafter "proxyl") and derivatives such as 3-(aminomethyl)-proxyl, 3-(2-[2-bromoacetamido]-acetamido)-proxyl, 3-(2-[2-2-bromoacetamido)-ethoxyethyl]-carbamoyl)-proxyl, 3-(2-bromoacetamido]-methyl)-proxyl, 3-(3-[2-bromoacetamido]-propylcarbamoyl)-proxyl, 3-(2-bromoacetamido)-proxyl, 3-carbamoyl-proxyl, 3-carboxy-proxyl, 3-cyano-proxyl, 3-(5-[dimethylamino]-1-naphthalene-sulfonamido)-proxyl, 3-(5-fluoro-2,4-dinitroanilino)-proxyl, 3-(2-[2-iodoacetamido]-acetamido)-proxyl, 3-(2-[2-(2-iodoacetamido)-ethoxyethyl]-carbamoyl)-proxyl, 3-(2-iodoacetamidomethyl)-proxyl, 3-(3-[2-iodoacetamido]-propylcarbamoyl)-proxyl, 3-(2-iodoacetamido)-proxyl, 3-(2-[2-isothiocyanatoethoxy]-ethylcarbamoyl)-proxyl, 3-(2-isothiocyanatoethylcarbamoyl)-proxyl, 3-(isothiocyanatomethyl)-proxyl, 3-(3-isothiocyanato-propyl carbamoyl)-proxyl, 3-(2-[2-maleimidoethoxy]-ethylcarbamoyl)-proxyl, 3-(2-maleimidoethyl-carbamoyl)-proxyl, 3-(maleimidomethyl)-proxyl, 3-(3-maleimidopropyl-carbamoyl)-proxyl, 3-maleimidoproxyl, 3-(4-nitrophenoxy carbonyl)-proxyl, N,N′-bis(3-proxyl carbonyl)-1,2-ethanediamine, and the like; 2,2,6,6,-tetramethyl-1-piperidinyloxyl (hereinafter "tempo") and derivatives such as 4-amino-tempo, 4-(2-bromoacetamido)-tempo, 4-[N-formyl-N-(3-hydroxypropyl)amino]-tempo, 4-[N,N-bis(2-hydroxyethyl)]amino-tempo, 4-(ethoxyfluorophosphinyloxy)-tempo, 4-hydroxy-tempo, 4-(2-iodoacetamido)-tempo, 4-isothiocyanato-tempo, 4-maleimido-tempo, 4-(4-nitrobenzoyloxy)-tempo, 4-oxo-tempo, 4-phosphonooxy-tempo, N,N′-bis(4-tempo)oxamide, 1,2-ethanediaminetetraacetic acid 2′,4′-bis[(CN-4-tempo)amide], N,N′-bis(4-tempo)-1,2-ethanediamine, N,N′-bis-(4-tempo)decanediamide, and the like; other spin labels such as 2-(acetoxymercuri)-4,4,5,5-tetramethyl-2-imidazolin-1-yloxy-3-oxide, 3-carbamoyl-2,5-dihydro-2,2,5,5-tetramethyl-1H-pyrrol-1-yloxy, 3,(-[ethoxycarbonyl]-oxycarbonyl)-2,5-dihydro-2,2,5,5-tetramethyl-1H-pyrrol-1-yloxy and the like; and nitrone and nitroso spin traps such as N-t-butyl-α-phenyl-nitrone, 3,5-dibromo-4-nitroso-benzenesulfonic acid, 5,5-dimethyl -1-pyrroline N-oxide, 2-methyl-2-nitroso-propane, nitrosobenzene, nitrosodisulfonic acid, α-(4-pyridyl-1-oxide)-N-t-butylnitrone, 3,3,5,5-tetramethyl-pyrroline N-oxide, 2,4,6-tri-t-butylnitrosobenzene, and the like; N,N¹-bis(4-tempo)oxamide, 1,2-ethanediamine tetra acetic acid 2¹,4¹-bis[(CN-4-tempo)amide], N,N¹-bis(4-tempo)-1,2-ethanediamine, N,N¹-bis(4-tempo)decanediamide. Such spin labels and spin traps are commercially available.

Other exemplary stable nitroxide radical-forming compounds include sodium azide, hydroxylamine, and the like. Particularly preferred nitroxide radical-forming compounds include, for example, nicotinamide N-oxide and nicotinic acid N-oxide, and the like.

Preferred stable sulfoxide radical-forming species include, for example, diazoxide and the related compounds described in U.S. Patents 2,896,572 and 4,184,039 which are hereby incorporated herein by reference. Diazoxide is 7-chloro-3-methyl-2H-1,2,4-benzo- thiadiazine 1,1-dioxide. Also contemplated as suitable hair growth stimulants in the composition are the substituted 1,2,4-benzothiadiazine 1,1-dioxides of the general formulae:



(I)



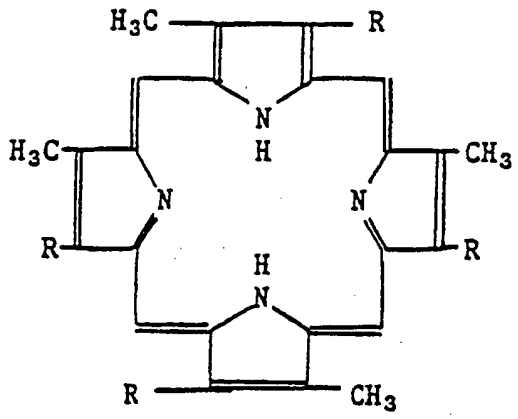
(II)

wherein X^2 is chlorine, bromine or trifluoromethyl in the 6, 7, 8 or 9 position or lower alkyl or lower alkoxy in the 6 position, and R^7 is alkyl, dialkylaminoalkoxyalkyl, or aralkyl, or a pharmacologically acceptable acid

addition salt thereof.

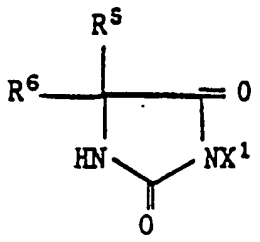
Specific representative examples of contemplated 1,2,4-benzothiadiazine 1,1-dioxides include:
3-methyl-7-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-ethyl-7-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-methyl-6-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-ethyl-6-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-n-pentyl-6-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-cyclopentyl-6-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-n-butyl-7-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-methyl-6-trifluoromethyl-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-methyl-6-trifluoromethyl-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3,6-dimethyl-7-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3,7-dimethyl-6-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-(2,4,4-trimethylpentyl)-6-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-octyl-6-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-dimethylaminoethoxymethyl-6-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-cyclohexenyl-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-heptyl-8-chloro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-styryl-8-chloro-3,4 dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide;
3-propyl-6-methyl-2H-1,2,4-benzothiadiazine 1,1-dioxide; and
3-methoxy-6-ethyl-2H-1,2,4-benzothiadiazine 1,1-dioxide.

Other stable free radical-forming compounds include the porphyrins, hydantoins and allantoins (5-ureido hydantoins). Porphyrins and physiologically active nitrogenous compounds, many of which occur naturally. Porphyrins are also known as substituted porphines and are derived from the following structure:



Specific representative examples of contemplated porphyrins include uroporphyrin, coproporphyrin, protoporphyrin and the like. Such compounds are typically complexed with transition metals such as iron, cobalt copper, and the like.

Hydantoins are particularly preferred stable free radical-forming hair growth stimulants and have the general formula:



wherein R^5 and R^6 are independently alkyl, aryl, alkaryl, haloaryl, alkoxyaryl, heteroaryl, aminoaryl or the like, or together are diarylene, and X^1 is hydrogen, alkali metal, alkaline earth metal, ammonium, alkylamine,

alkanolamine, polymethylene diamine or the like. Specific representative examples include 5,5-diphenyl-hydantoin, 5-phenyl-5-(p-bromophenyl)-hydantoin, 5-phenyl-5-(p-chlorophenyl)-hydantoin, 5,5-di-(p-dimethylaminophenyl)-hydantoin, 5-diphenylene-hydantoin, 5-xylenyl-5-phenylhydantoin, 5-ureidohydantoin, 5,5-(di-p-tolyl)-hydantoin, 5-phenyl-5-anisylhydantoin, 5-phenyl-5-(2-thienyl)-hydantoin, sodium salts thereof and the like. Such compounds and their preparation are described, for example, in U.S. Patents 2,366,221 and 2,409,754, which are incorporated herein by reference.

Other exemplary stable radical formers contemplated herein include p-phenolsulfonate, phenozine methosulfate, 7,7,8,8-tetracyanoquinodimethane, and the like.

Radical generating species reactive with reducing agents to form stable free radicals such as nitric oxide and peroxide, generally include hydroxylamine, organic nitrates, inorganic nitrites, peroxides, and the like. The organic nitrates and inorganic nitrites are preferred source radical compounds. Exemplary organic nitrates include trinitroglycerin, amyl nitrate, erithrithyl tetranitrate, isosorbide dinitrate, isosorbide-2-nitrate, isosorbide-5-nitrate, isomannide dinitrate, isomannide 2-nitrate, isoidide dinitrate, isoidide-2-nitrate, sodium nitruprosside and the like. Inorganic nitrites generally include alkali and alkaline earth metal nitrates and transition metal nitrates, such as, for example, sodium nitrite, potassium nitrate, lithium nitrite, and the like. Contemplated peroxides include benzoyl peroxide, hydrogen peroxide, urea hydrogen peroxide, carbamoyl peroxide, and the like.

The adjuvant is selected from hydroxyl radical scavengers, reducing agents, antioxidants and antiandrogens. The adjuvant used in the present method serves to induce formation of the free radical species of, or from, the foregoing compounds, to stabilize the free radicals once formed, and/or to protect the desired free radicals from reacting with other free radical species present in the skin, e.g. by scavenging or inhibiting formation of hydroxyl or other radicals with which the radical-forming compounds, or the radical formed therefrom, is reactive. Regardless of the mechanism involved, the adjuvant synergizes with the free radical-forming compound to enhance the hair growth stimulation thereof on repeated topical applications.

The type of adjuvant employed will depend on the particular type of free radical-forming compound employed. One class of adjuvants preferred for use in conjunction with the stable free-radical forming compounds described above is the group of compounds including radical scavengers, and particularly the pharmacologically suitable reducing agents and antioxidants which are generally effective hydroxyl radical scavengers.

As used herein, the term "free radical scavenger" includes compounds which suppress free radical generation as well as compounds which react with free radicals in biological systems. Hydroxyl radicals scavengers are, for example, sulfoxides, phosphine oxides, retinoids, purines, pyrimidines, thiols, halide ions, aromatic hydrocarbons and the like. Free radical scavengers suitable in the present invention include those pharmacologically acceptable hydroxyl radical scavengers which have a substantial effectiveness as a hydroxyl radical scavenger, preferably compounds having an effectiveness as a hydroxyl radical scavenger substantially equivalent to or better than DMSO, i.e. a specific reaction rate constant with hydroxyl radical on the order of 5-6 x 10^9 dm^3/mol-sec or higher, and particularly hydroxyl radical scavengers having a higher reactivity with hydroxyl radicals than nitric oxide, i.e. on the order of 8.9-11x10^{10} dm^3/mol-sec or more.

One preferred class of reducing agents and free radical scavengers includes sulfoxides of the formula R^8R^9SO wherein R^8 is alkyl, alkenyl, heteroalkyl (e.g. thiaalkyl or azaalkyl), hydroxyalkyl, or alkoxyalkyl having up to about 14 carbon atoms, and R^9 is independently alkyl or hydroxyalkyl having from 1 to about 8 carbon atoms. Examples of R^8 suitable herein include octyl, nonyl, decyl, undecyl, dodecyl, 3-decenyl, 2-dodecenyl, 3-undecenyl, 3-octenyl, 2-ketooctyl, 2-ketodecyl, 2-ketoundecyl, 2-ketododecyl, 2-hydroxyoctyl, 2-hydroxydecyl, 2-hydroxyundecyl, 2-hydroxydodecyl, 3-hydroxyundecyl, 3-methoxyundecyl, 2-methoxydodecyl, 3,6-dioxadodecyl, 2-ethylhexyl, and branched chain nonyl and dodecyl resulting from polymerization of three and four moles of propylene, respectively, and the like. Examples of R^9 include methyl, ethyl, propyl, butyl, hydroxymethyl, 2-hydroxyethyl, 3-hydroxypropyl, and 4-hydroxybutyl, and the like.

Especially preferred sulfoxides for the purposes of this invention are the dialkyl sulfoxides where R^8 is a hydrocarbyl alkyl or hydroxy-substituted alkyl group containing from 8 to 12 carbon atoms and R^9 is methyl, ethyl or propyl. As examples of these preferred sulfoxides there may be mentioned octyl methyl sulfoxide, nonyl methyl sulfoxide, decyl methyl sulfoxide, undecyl methyl sulfoxide, dodecyl methyl sulfoxide, 2-hydroxydecyl methyl sulfoxide, 2-hydroxyundecyl methyl sulfoxide and 2-hydroxydodecyl methyl sulfoxide.

Another preferred class of reducing agents and hydroxyl radical scavengers includes the tertiary phosphine oxides of the formula $R^{10}R^{11}R^{12}PO$ wherein R^{10} is alkyl, aralkyl heteroalkyl (e.g. azaalkyl or thiaalkyl), hydroxyalkyl, alkoxyalkyl, or ketoalkyl of from 1 to 14 carbon atoms, or aryl of from 6 to 12 carbon atoms, and R^{11} and R^{12} are independently alkyl, hydroxyalkyl, alkoxyalkyl or ketoalkyl of from 1 to 4

carbon atoms. Examples of R^{10} incude methyl, ethyl, propyl, butyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, 2-propenyl, 3-decenyl, 2-dodecenyl, 3-undecenyl, 3-octenyl, 2-ketobutyl, 2-ketohexyl, 2-ketooctyl, 2-ketodecyl, 2-ketoundecyl, 2-ketododecyl, 2-hydroxypropyl, 2-hydroxyhexyl, 3-hydroxyheptyl, 2-hydroxyoctyl, 2-hydroxyundecyl, 2-hydroxydodecyl, 3-hydroxyundecyl, 2-methoxybutyl, 3-methoxyundecyl, 2-methoxydodecyl, 2-chlorodecyl, 3-chlorobutyl, 2-thiomethylhexyl, 3,6-dioxadodecyl, 2-oxaheptyl, 3-azahexyl, 2-thiadecyl, 2-ethylhexyl, phenyl, naphthyl, m-tolyl, benzyl, and branched chain nonyl and dodecyl resulting from polymerization of three and four moles of propylene, respectively.

Examples of R^{11} and R^{12} include methyl, ethyl, propyl, hydroxymethyl, 1-hydroxypropyl, 2-hydroxyethyl, and the like.

Especially preferred phosphine oxides for the purpose of this invention are those in which R^{10} is a hydrocarbyl alkyl or hydroxy-substituted alkyl substituent containing from 8 to 12 carbon atoms and R^{11} and R^{12} are each methyl, ethyl or propyl. As examples of these preferred phospine oxides there may be mentioned octyl dimethyl phosphine oxide, nonyl diethyl phosphine oxide, decyl dimethyl phosphine oxide, undecyl dimethyl phosphine oxide, dodecyl dimethyl phosphine oxide, 2-hydroxydecyl dimethyl phosphine oxide, 2-hydroxyundecyl dimethyl phosphine oxide and 2-hydroxydodecyl dimethyl phosphine oxide. Dodecyl dimethyl phosphine oxide is especially preferred.

The retinoids comprise another preferred class of free radical scavengers and reducing agents. Exemplary retinoids include carotene, tretinoin, isotretinoin, 9-cis-tretinoin, retinol, retinol acetate, retinol palmitate, dehydroretinol, 9-cis-dehydroretinol, 13-cis-dehydroretinol, 9,13-di-cis-dehydroretinol, retinal, etretinate, retinyl acetate, 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6, 8-nonatetraenoic acid and the like. Especially preferred retinoids include tretinoin and 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid.

Other contemplated adjuvants include, for example, mannitol, benzoic acid, p-aminobenzoic acid, thioproline, homocysteine, cysteine, acetyl cysteine, dithiothretiol, sodium dithionate, thiosalicyclic acid, mercaptoethanol, ascorbate, thiodipropionic acid, dilauryl thiodipropionate, mercaptoethanolamine, propyl gallate, hydroxyethylruticide, pantothenic acid, and the like. Other suitable adjuvants may be selected from pharmacologically acceptable compounds which have substantial reactivity with hydroxyl radicals, such as those listed in Ross and Ross, "Selected Specific Rates of Reactions of Transients from Water in Aqueous Solution. III. Hydroxyl Radical and Perhydroxyl Radicals and Their Radical Ions", National Standard Reference Data Series, National Bureau of Standards, 59 (1977), which is incorporated herein by reference, and preferably those compounds having a specific rate (k) of at least 5×10^9, and especially at least 10^{10}.

Another class of adjuvants contemplated as useful herein include antioxidants such as superoxide dismutase (SOD) and compounds having SODase activity, such as, for example, copper aspirinate, indomethocin-copper, and the like. Generally, about 300 units per application is contemplated as an effective amount, but more or less than this may be employed. Also contemplated are basic amino acids, peptides containing basic amino acids (particularly peptides comprised predominantly or entirely of basic amino acids), and metal complexes thereof, such as, for example, glycine, histidine, lysine, arginine, cysteine, methionine, histidyl lysine, glycyl histidine, glycyl hystidyl lysine, lysyl histidyl lysine, etc. Copper, zinc, manganese and iron and other transition metal complexes of these amino acids possess superoxide dismutase activity.

Other compounds which have been found to be helpful as adjuvants in enhancing the hair growth stimulating activity of the free radical-forming compounds include antiandrogens, preferably those which interfer with the binding of androgens such as dihydrotestosterone to receptors in hair follicles. However, antiandrogens which interfere with or inhibit the synthesis of androgenic compounds are also contemplated. Although not fully understood, the preferred antiandrogens appear to function primarily to block dihydrotestosterone receptors rather than to inhibit the reduction of testosterone, and are also known to DHT blockers, but it is also possible that such antiandrogens may function as free radical formers, reducing agents or hydroxyl radical scavengers. Exemplary of such antiandrogens are spironolactone, cyproterone, cyproterone acetate, and the like. Of these, spironolactone is preferred because its effects from topical application are generally more limited to the local site of application.

Effective amounts of the antiandrogen generally range from about 0.01 to about 5 percent by weight of the preparation(s) applied, but more or less than this may be used depending on the particular antiandrogen. The optimum amount is about one percent by weight of the preparation(s) for spironolactone and about 0.1 percent for cyproterone and cyproterone acetate.

In one embodiment of the method, the free radical source and the adjuvant are applied as a premixed topical preparation. The free radical source compound in this embodiment should be relatively stable in the mixture, and the adjuvant, therefore, must be essentially chemically unreactive with the source compound, i.e. to the extent that the mixture contains or remains capable of forming free radicals for a reasonable

shelf-life. Preferably, the preparation contains a stable free radical-forming compound, especially a nitroxide radical former, at a suitable concentration, e.g. 10-100 mM, and a reducing agent, antioxidant or hydroxyl radical scavenger at a similar concentration. In general, hydroxyl radical scavengers may in some instances be used as solvents at relatively high concentrations. Antioxidants are generally active at trace concentrations, e.g. as low as 0.02 weight percent or lower. As typical examples, there may be mentioned premixed combinations of minoxidil with dimethyl sulfoxide, tretinoin, cysteine, p-aminobenzoic acid, dodecyl dimethyl phosphine oxide, cystein and/or SOD and the like; diazoxide with octyl methyl sulfoxide, octyl dimethyl phosphine oxide, 9-(4-methoxy-2,3,6-trimethylphenyl)-3, 7-dimethyl-2,4,6,8-nonatraenoic acid, thiodopropionic acid and/or copper aspirinate and the like; protoporphyrin with DMSO, trimethyl phosphine oxide, retinol, histidine, dithiothreitol and/or SOD and the like; 5-5-diphenylhydantoin with DMSO, hydroxyethyl dimethyl phosphine oxide, retinol palmitate, pantothenic acid, and/or SOD and the like; nicotinamide N-oxide or nicotinic acid N-oxide with ethanol, DMSO, propyl dimethyl phosphine oxide, tretinoin, sodium dithionate, lyslyl-histidyl lysine, and/or copper aspirinate, and the like; and other similar combinations of the above mentioned stable radical formers with one or more of the above mentioned adjuvants. Particularly preferred are combinations of a stable radical former with a hydroxyl radical scavenger and a compound with SODase activity. Such combinations of ingredients are generally effective to stimulate hair growth when used as hereinbefore described. In addition, the preparation containing the stable radical former, the hydroxyl radical scavenger and any SODase activity, may also include an antiandrogen.

In some instances, the stable radical former may be a sufficiently effective hair growth stimulant that no adjuvant need be employed, i.e. such stable radical formers may be used without a hydroxyl radical scavenger, or with a relatively weak hydroxyl radical scavenger. Inasmuch as such compounds are previously unknown for topical use as hair growth stimulants, they are within the purview of the present invention. Contemplated examples of these include nicotinic acid N-oxide, nicotinamide N-oxide, tempo, proxyl, doxyl, N-t-butyl-α-phenyl nitrone and the like. However, the activity of the stable radical former is generally enhanced or potentiated by combination with an antioxidant, reducing agent or hydroxyl radical scavenger, and such combinations are therefore preferred.

In another embodiment of the method of the invention, a free radical forming or generating compound preferably in a pharmaceutical carrier, is admixed witth a reducing agent, also preferably in a pharmaceutical carrier, at or near the time of application to the skin. The radical formers reactive with reducing agents to generate free radicals are preferred, and especially the free radical-forming compounds which react with reducing agents to form nitric oxide. Organic peroxides can also be used to generate free radicals upon reaction with reducing agents. Free radical forming compounds, such as, for example, organic nitrates and inorganic nitrites, react with reducing compounds to form nitric oxide radicals as a reaction product. However, the formation of nitric oxide is generally irreversible since the nitric oxide radical is itself relatively reactive with a half life of about 30 seconds. Thus, it is important in this embodiment of the method to separate the nitric oxide radical-forming compound from the reducing agent until use. Just before application, the nitric oxide radical-forming compound is admixed with the activating agjuvant to initiate formation of the nitric oxide. Then, while the nitric oxide reactants are still being produced by the reaction between the nitrate or nitrite and the reducer, the admixture is applied to the skin at which hair growth stimulation is desired. Alternatively, the nitrate or nitrite may be applied directly to the skin in a first pharmaceutical carrier, and the reducing compound applied in a second pharmaceutical carrier. The order of application is not critical, as long as the nitrate or nitrite reacts with the reducer on or in the skin to release nitric oxide therein, although it may be advantageous to allow a period of time for either the nitric oxide source compound or the reducer to penetrate into the skin so that more nitric oxide is formed in the skin adjacent the hair follicles rather than on the skin surface where the nitric oxide may be released into the ambient air with the result of less effective hair growth stimulation. A period of time of about 15-30 minutes between application of the nitrate or nitrite and the reducer is generally sufficient, although the optimum time may depend on the particular nitrate or nitrite, adjuvant, carrier and skin condition, as these variables may affect skin penetrability. If desired, a compound with SODase activity and/or an antiandrogen may also be present in either preparation, preferably the preparation containing the nitric oxide generating compound, to further enhance the hair growth stimulation.

Another aspect of the invention is the provision of a kit for concurrently measuring and applying a unit dosage of the free radical source compound and an adjuvant. The kit includes a unit dose of the free radical source compound, a unit dose of the adjuvant, means for maintaining the unit doses in an unmixed or uncombined state, and means for dispensing the unit doses. The free radical source compound and the adjuvant are generally in association with respective first and second topical pharmaceutical carriers, which may be the same or different carriers. Preferably, the free radical source compound is capable of generating nitric oxide, such as, for example, an organic nitrate or an inorganic nitrate, and the adjuvant is a

reducing agent reactive with the free radical source compound to form the nitric oxide. Organic peroxides may also be used as the free radical source. If desired, a compound with SODase activity and/or an antiandrogen are included in either unit dose, preferably in the unit dosage of the nitrate or nitrite. The kit optionally further includes means for mixing the nitrate or nitrite unit dose with the unit dosage of the reducing agent.

With reference to specific embodiments of the present kit, one version includes a conventional packaging container such as a box or carton containing a plurality of individually sealed packets of 0.5-1 ml of 0.01-5 weight percent free radical source and any SODase and/or antiandrogen in a suitable carrier, and a like plurality of individually sealed packets of 0.5-1 ml of 10-100 mm reducing agent in a suitable carrier. The packets are foil, plastic, coated paper or the like and are manufactured, filled and sealed as is conventional in the pharmaceutical and cosmetic arts. Preferably, the packets are marked with visual indicia to indicate the contents thereof, i.e., either a "step 1" or a "step 2" packet. Conveniently, each box or carton contains a supply of packets for a series of applications for one or two weeks or a month, for example. In use, a packet of each of the free radical source and reducing agent are broken or torn open, dispensed from the packet by pouring or squeezing as appropriate, mixed in the palms of the hands, and the mixture is then applied to the scalp or other area of treatment. If desired, a small cup may be included in the kit for mixing the unit dose of the free radical source with that of the reducing agent. Alternatively, the unit dose of free radical source is applied first to the scalp, followed by the unit dose of the reducing agent, or vice versa. In another version of this kit, the packets comprise three layers of foil or other membraneous material, one interposed between the other two to form two chambers on either side thereof. The free radical source unit dosage, its respective carrier and any SODase activity and/or antiandrogen are placed in one chamber, and the reducing agent and its respective carrier are placed in the other chamber. When the packet is broken or opened, both chambers are simultaneously dispensed and mixed prior to application, such as, for example, by squeezing the packet to extrude the unit dosages and empty the chamber contents into the palm of the hand.

In an alternative kit embodiment, dual reservoirs for the two preparations are commonly dischargable so that proportionate amounts of each may be dispensed, preferably through a common discharge point. For example, a syringe with a chamber divided into two reservoirs can be employed with a split plunger for discharging a unit dose of each preparation may be employed, or a hand pump with split suction tubes extending into each of the reservoirs.

The invention is also a topical preparation for stimulating hair growth from skin upon repeated application. Broadly, the preparation includes a stable free radical source compound, preferably a nitoxide spin label or spin trap as described hereinabove, in the pharmaceutical carrier also described hereinabove. The free radical source compound may be at a concentration of 0.1 to 20 percent by weight, preferably 0.5 to 3 percent by weight, e.g. 10-100 mM, but more or less than this can be used in some instances for suitable results, depending on the particular free radical source.

In a preferred embodiment, the topical preparation also includes adjuvant. The adjuvant may be a reducing agent, a hydroxyl radical scavenger, an antioxidant, or a combination thereof, and may also include an antiandrogen, all as described hereinabove. The reducing agent and hydroxyl radical scavenger may be present in the preparation at a concentration similar to that described above for the free radical source, i.e. generally at 0.1-20 weight percent, preferably 1-3 weight percent, although higher concentrations could also be used, while antioxidants such as those with SODase activity are generally present at much lower concentrations, e.g. 0.01-10 mM, or a sufficient amount to provide about 300 units of SODase activity.

In one particular embodiment of the preparation, minoxidil or a suitable substitute therefor is preferably present in the composition in an amount of from about 0.1 to about 20 percent by weight of the composition, more preferably from about 0.5 to about 3 percent by weight, and most preferably about 2 percent by weight. For convenience, reference is made in this embodiment to minoxidil, but it is to be understood that the suitable substitutes therefor described above may be present partially or entirely in lieu of minoxidil itself. The second essential ingredient in this embodiment is DMSO or a substantially equivalent hydroxyl radical scavenger in a synergistically effective amount. Hydroxyl radical scavengers are, for example, sulfoxides, purines, pyrimidines, thiols, alcohols, halide ions, aromatic hydrocarbons and the like. Hydroxyl radical scavengers suitable in the composition of the present embodiment are those pharmaceutically acceptable hydroxyl radical scavengers which have a hydroxyl radical scavenger effectiveness substantially equivalent to or better than DMSO. Preferred hydroxyl radical scavengers are the alkyl methyl sulfoxides in which the alkyl substituent has from one to about 14 carbon atoms and the β-hydroxyalkyl methyl sulfoxides in which the hydroxyalkyl substituent has from two to about 14 carbon atoms, with dimethyl sulfoxide being particularly preferred. Specific representative examples of such sulfoxides include,

in addition to DMSO, hexyl methyl sulfoxide, decyl methyl sulfoxide, dodecyl methyl sulfoxide, tetradecyl methyl sulfoxide, β-hydroxydecyl methyl sulfoxide, β-hydroxydodecyl methyl sulfoxide, β-hydroxytetradecyl methyl sulfoxide, and the like. For convenience, reference is made hereinbelow to DMSO, but it is to be understood that other suitable hydroxyl radical scavengers may be present, partially or entirely, in lieu of DMSO. The hydroxyl radical scavenger is present in the composition in a proportion effective to, synergistically with the minoxidil, stimulate the growth of hair. For the sulfoxides such as DMSO, this amount is generally from about 5 to about 25 percent by weight of the composition, preferably about 15-20 percent by weight. Depending on the particular carrier, the amount of DMSO present may be adjusted to avoid phase separation. It has also been found that the hair growth stimulation effected in this embodiment is further improved when an antiandrogen as described above is present.

The mechanism for the hair growth stimulation achieved with stable free radicals and antioxidants, hydroxyl radical scavengers and reducing agents is not fully understood, and the invention is not to be constrained or limited by theory. It is believed that stable free radicals, especially nitroxide radicals, are mechanistically involved in stimulating follicle cells to grow hair. The reducing agents are believed to enhance the activity of the free radicals by stimulating their formation, and also by functioning as hydroxyl radical scavengers. The hydroxyl radical scavengers are believed to protect the stable radicals in vivo by reducing the concentration of hydroxyl radicals, thereby inhibiting the inactivation of the stable free radicals by the hydroxyl radicals with which they are extremely reactive. Antioxidants such as SOD similarly protect the stable radicals since they cause the dismutation of superoxide which is known to be involved in the formation of hydroxyl radicals. The antiandrogens could be acting to inhibit the adverse effects of DHT on hair growth, but could also be functioning as hydroxyl radical scavengers. Inasmuch as nitric oxide is known to mimic the vasodilating activity of organic nitrates by stimulating the enzyme guanylate cyclase as reported, for example, in Feelisch et al, European Journal of Pharmacology, vol. 139, pp. 19-30 (1987), it is contemplated that other compounds such as cyclic GMP are also potent hair growth stimulants, and that repeated topical application of effective amounts of cyclic GMP in association with a pharmaceutical carrier can stimulate hair growth at the site of application.

The preparation and use of the composition is illustrated by way of the following examples.

Example 1

A composition according to the invention was prepared with the ingredients and proportions listed in Table I.

Table I

Ingredient	Proportion
Dermovan emulsion[1]	15 pounds
DMSO	3 pints
Water	2 pints
Propylene glycol	2 pints
Diphenyl hydantoin	0.5 wt.%
Spironolactone	0.5 wt.%
Notes for Table I:	

1. Obtained from Owen Laboratories; Dermovan emulsion contains water, glycerol stearate, glycerin, mineral oil, synthetic spermaceti, cetyl alcohol, butylparaben, propylparaben and methylparaben.

The water and propylene glycol were added to the diphenyl hydantoin in a suitable container. The DMSO was then added and the mixture was thoroughly mixed and allowed to stand overnight. Then, with constant stirring the Dermovan emulsion was added slowly. The mixture was then allowed to stand at least 24 hours with occasional stirring.

Example 2

A composition is prepared as in Example 1 except that 2.0 percent by weight of sodium diazoxide is substituted in place of the diazoxide and the proportion of spironolactone is decreased to 0.01 percent by weight.

Example 3

A topical gel was prepared with the following ingredients and proportions:

Table II

Ingredient	Proportion
DMSO	3 pints
Propylene glycol	3 pints
Water	3 pints
Spironolactone	1 wt.%
Diphenyl hydantoin	1 wt.%
Hydroxypropyl cellulose (M.W. 100,000-1,000,000)	1 wt.%

The ingredients were combined with stirring and allowed to sit for 3-5 days until the mixture formed a gel.

Example 4

A lotion was prepared with the following ingredients and proportions:

Table III

Ingredient	Proportion
Propylene glycol	2 pints
Water	2 pints
Ethyl alcohol	6 pints
Urea	10 wt.%
Spironolactone	1 wt.%
Diphenyl hydantoin	1 wt.%

The ingredients were combined with stirring to form a lotion.

Example 5

A cream was prepared as in Example 1, except that 1 pint of propylene glycol was used instead of 2 pints, 1 wt.% minoxidil was used instead of diphenyl hydantoin, 1 wt.% spironolactone instead of 0.5 wt.%, and the cream also contained 0.01 wt.% tretinoin added with the minoxidil and spironolactone.

Example 6

The composition of Example 1 was applied topically to the scalps of male patients with 2-5 years of hair

loss who had all been previously treated with 2 wt.% minoxidil in a solution of water (70 vol.%), ethanol (15 vol.%) and propylene glycol (15 vol.%) without any significant promotion of hair growth. The composition of Example 1 was applied to the scalp twice daily at a rate of 1 ml/day. About half of the subjects responded with photographically verifiable hair growth after 2-6 months of treatment. In contrast, a control group similarly administered the composition of Example 1, but without any diphenyl hydantoin, exhibited less hair growth and had a longer response time, although the number of subjects eventually responding was also about half of the group.

Example 7

A lotion is prepared by combining the following proportion of ingredients:

TABLE IV

Ingredient	Proportion
Nicotinamide N-oxide	1 g
Ascorbyl palmitate	0.1 g
Spironolactone	0.5 g
Decylmethyl sulfoxide	5 g
Propylene glycol	30 ml
Ethanol	20 ml
Distilled water	45 ml

The preparation is applied at a rate of about 0.5-1 ml one to three times per day to the affected area until hair growth is stimulated in about three months.

Example 8

A composition is prepared as in Example 1 using the following proportions of ingredients:

TABLE V

Ingredient	Proportion
Nicotinic acid N-oxide	1 g
2-Pyrrolidone	5 g
Dermovan emulsion	75 g
Distilled water	20 g

Example 9

A composition is prepared as in Example 1 using the following proportions of ingredients:

TABLE VI

Ingredient	Proportion
Protoporphyrin IX, disodium salt	1 g
Cysteine	1 g
N-methyl-2-pyrrolidone	5 g
DMSO	20 g
Distilled water	20 g
Dermovan emulsion	60 g

Example 10

A composition is prepared as in Example 1 with the following proportions of ingredients:

TABLE VII

Ingredient	Proportion
Allantoin	2 g
DMSO	20 ml
Spironolactone	1 g

Example 11

A first composition is prepared containing benzoyl peroxide (5g), distilled water (20g), ethanol (30g) and propylene glycol (50g); and a second composition containing retonoic acid (0.05 g) and Dermovan emulsion (100 g). The compositions are separated until use. The first composition is applied to the affected area at 0.25-0.5 ml per application along with a like volume of the second composition. The applications are made 1-3 times per day until hair growth stimulation is seen at about three months.

Example 12

Aqueous sodium nitrile at 0.6 weight percent is combined with 2 weight percent cysteine and 0.5-1.0 ml of the mixture is immuliately applied to the affected area. The procecdure is repeated 1-3 times per day, and stimulated hair growth is observed at about 3 months.

Example 13

A lotion is made by homogenizing the following proportions of ingredients:

TABLE VIII

Ingredient	Proportion
Copper asprinate	0.1 g
Ascorbyl palmitate	0.5 g
Dermovan emultion	100 g

The composition is used as in Example 7.

Example 14

Example 13 is repeated, but using the following proporations of ingredients:

TABLE IX

Ingredient	Proportion
N-t-butyl-α-phenyl nitrone	0.1 g
Spironolactone	0.5 g
BHT	0.01 g
DMSO	20 ml
Water	20 ml
Dermovan emulsion	60 g

Example 15

Example 13 is repeated, but using the following ingredients

TABLE X

Ingredient	Proportion
Lysyl-histidyl-lysine	50 mg
Cupric chloride	50 mg
Spironoactone	0.5 g
Water	30 ml
Propylene glycol	30 ml
Ethanol	20 ml

Example 16

Example 13 is repeated, but using the following proportions of ingredients:

TABLE XI

Ingredient	Proportion
Sodium 3,5-dibromo-4-nitrosobenzene sulfonate	0.1 g
Spironolactone	0.5 g
Retinoic acid	0.05 g
Water	20 ml
BHT	0.05 g
Propylene glycol	30 g
Ethanol	50 g

Example 17

Example 13 is repeated, but using the following proportions of ingredients:

TABLE XII

Ingredient	Proportion
Phenytoin	2 g
Sodium benzoate	1 g
Spironolactone	1 g
Water	30 g
Dermovan emultion	70 g

Example 18

Example 13 is repeated, but using the following proporation of ingredients:

TABLE XIII

Ingredient	Proportion
Phenytoin	2 g
Sodium benzoate	1 g
Spironolactone	1 g
Water	30 g
Dermovan emulsion	70 g

Example 19

Example 18 is repeated, but no spironolactone is used and 0.5 g thiodipropionic acid is used in place of the sodium benzoate.

Example 20

The following proportions of ingredients are combined as in Example 1:

TABLE XIV

Ingredient	Proportion
Minoxidil sulfate	1 g
Dilauryl thiodipropionate	0.5 g
Water	20 ml
Propylene glycol	20 ml
Dermovan emulsion	60 g

Example 21

A lotion was prepared as in Example 1 with the following proportions and ingredients:

TABLE XV

Ingredient	Proportion
Minoxidil	0.1 g
Spironolaetone	1 g
Phenytoin	2 g
Ascorbyl palmite	0.03 g
Retinoic acid	0.05 g
BHT	0.02 g
DMSO	20 ml
Water	20 ml
Dermovan emulsion	60 g

This lotion was topically applied at 0.5 ml once a day for hair growth stimulation.

Example 22

A composition according to the invention was prepared with the following ingredients and proportions:

Table XVI

Ingredient	Proportion
Dermovan emulsion	15 pounds
DMSO	3 pints
Water	2 pints
Propylene glycol	2 pints
Minoxidil	2 wt.%

The water and propylene glycol were added to the dry minoxidil in a suitable container. The DMSO was then added and the mixture was thoroughly mixed and allowed to stand overnight. Then, with constant stirring the Dermovan emulsion was added slowly. The mixture was then allowed to stand at least 24 hours with occasional stirring.

Examples 23-25

Other compositions according to the invention were prepared with the ingredients and proportions listed in Tables XVII through XIX. The compositions were prepared as in Example 22 with any spironolactone being added with the minoxidil.

Table XVII

Ingredient	Proportion
Dermovan emulsion	15 pounds
DMSO	3 pints
Water	2 pints
Propylene glycol	2 pints
Minoxidil	2 wt.%
Spironolactone	0.01 wt.%

Table XVIII

Ingredient	Proportion
Dermovan emulsion	15 pounds
DMSO	3 pints
Water	2 pints
Propylene glycol	2 pints
Minoxidil	1 wt.%
Spironolactone	0.01 wt.%

Table XIX

Ingredient	Proportion
Dermovan emulsion	15 pounds
DMSO	1 pint
Water	2 pints
Propylene glycol	2 pints
Minoxidilq	2 wt.%
Spironolactone	0.01 wt.%

Example 26

The composition prepared according to the procedure of Example 22 was used to treat the balding scalp area of a 27-year-old male patient with 9 years of hair loss, most of which had occurred during the 4 years preceding treatment. The patient had recently been treated in a formal clinical trial conducted by The Upjohn Company with topical minoxidil, believed to be 2 wt.% minoxidil in a solution of water (70 vol. %), ethanol (15 vol. %) and propylene glycol (15 vol. %), applied to the scalp three times a day for over a year with no effect. The composition of Example 22 was applied once a day to the water-soaked scalp, e.g. immediately following bathing, at a rate of about 0.5 ml per day. Visually perceptible hair growth at the previously bald areas of the scalp was observed after about 2 months of treatment. Nearly normal hair growth was observed at about 4-6 months of treatment.

Example 27

The composition of Examples 22, 23 and 24 were used to treat the balding scalp area of a 34-year-old male with 3-4 years of hair loss. The patient had previously been treated with topical minoxidil as described in Example 26, with no substantial results. The composition of Example 22 was applied to the scalp as in Example 26 for about 3 months, then the composition of Example 23 for about 1 month, and thereafter the composition of Example 24. The patient responded with visibly improved hair growth at about 2 months of treatment which continued to improve at six months.

Example 28

The composition of Example 22 was used to treat the balding scalp area of a 26-year-old male with about 4 years of hair loss. The patient had previously been treated with topical minoxidil as described in Example 26 with no substantial results. The composition of Example 22 was applied to the scalp as in Example 26. By one month, the patient's hair line recession had stopped and there was a marked decrease in hair loss. At about 3 months, visibly improved hair growth was observed with forward movement of the hair line. Improvement in hair growth has continued.

Example 29

The composition of Examples 22 and 25 were used to treat the balding scalp area of a 42-year-old male with about 12 years of hair loss. The patient had previously been treated with topical minoxidil as described in Example 26, with no substantial results. The composition of Example 22 was applied to the scalp as in Example 26 for about 4 months, and thereafter the composition of Example 25. The patient showed visibly improved hair growth beginning at two months of treatment, which continued.

Example 30

The composition of Example 24 was used to treat the balding scalp area of a 26-year-old male with 1 year of hair loss. The patient has previously been treated with topical minoxidil as described in Example 26, with no substantial results. The composition of Example 25 was applied to the scalp as in Example 26. The patient showed decreased hair loss at one month with gradual replacement and marked forward progression of the hairline at four months, which continued.

Example 31

The composition of Example 22 was used to treat the balding scalp area of a 33-year-old male with about 5 years of hair loss. The patient had been previously treated with topical minoxidil as described in Example 26 with no substantial results. The composition of Example 22 was applied to the scalp as in Example 26. Improved hair growth was visible at two months and markedly so at three months. Improvement in hair growth continued.

Example 32

The composition of Examples 22, 23 and 24 were used to treat the balding scalp area of a 34-year-old female with about 3-1/2 years of hair loss secondary to alopecia areata. The patient had previously been treated with topical therapy using chlorodinitrobenzene sensitization, with no substantial results. The

composition of Example 22 was applied to the scalp as in Example 26 for about 4 months, then Example 23 for about 1 month, and Example 24 thereafter. The patient showed significantly decreased hair loss and improved growth of hair at two months. White hair was thickened and darkened with visible results present by three months. Progress has continued.

Example 33

A 27-year-old male patient with about 5 years of hair loss from the scalp experienced local allergic dermatitis about 3 months after initial application of the composition of Example 22 to the scalp as in Example 26. The application of this composition was discontinued, and topical application of 2 wt.% minoxidil in an alcohol-based carrier (70 vol.% water, 15 vol.% ethanol, 15 vol.% propylene glycol) was started without any allergic reaction. After about 2 months, application of the minoxidil in the alcohol-based carrier was discontinued, and application of the composition of Example 22 started. Throughout treatment, there was no allergic reaction to either the Minoxidil in the alcohol-based carrier, or to the composition of Example 22 following sensitization with the minoxidil in the alcohol-based carrier. Improved hair growth was observed at about 2 months following initial application of minoxidil, and has continued.

Example 34

The composition of Example 21, but without minoxidil, has also been used as a topical scalp medicament.

Claims

1. A method for stimulating hair growth, comprising:
topically applying the skin a pharmacologically acceptable nitroxide radical-forming compound; and
concurrently therewith, topically applying to the skin an adjuvant selected from reducing agents, antioxidants and hydroxyl radical scavengers.

2. A method according to claim 1, wherein applications are repeated over a sufficient period of time to stimulate hair growth.

3. A method according to claim 1 or 2, wherein the radical-forming compound and the adjuvant are separated from each other until application.

4. A method according to claim 1 or 2, wherein the radical-forming compound and the adjuvant are intermixed immediately prior to application.

5. A method according to any of claims 1 to 4, wherein the adjuvant has SODase activity.

6. A method according to any of claims 1 to 5, wherein the radical-forming compound and adjuvant are in association with respective pharmaceutical carrier or carriers.

7. A method for stimulating hair growth, comprising:
mixing, in a pharmaceutical carrier (i) a pharmacologically acceptable stable nitroxide radical-forming compound with (ii) an amount of pharmacologically acceptable reducing agent effective to induce nitroxide radical formation from the stable compound;
applying the nitroxide-forming mixture topically to skin; and
repeating application over a period of time sufficient to stimulate hair growth from the skin.

8. A method according to claim 7, wherein the radical-forming compound and the reducing agent are separated prior to mixing, and the mixture is applied to skin within about one hour following mixing.

9. A method according to claim 7 or 8, wherein the nitroxide radical-forming compound is trinitroglycerin, amyl nitrate, erithrithymyl tetranitrate, isosorbide dinitrate, isosorbide-2-nitrate, isosorbide-5-nitrate, isomannide dinitrate, isomannide-2-nitrate, isoidide dinitrate, or isoidide-2-nitrate.

10. A method according to claim 7 or 8, wherein the nitroxide radical-forming compound is an organic nitrate or an inorganic nitrite.

11. A method according to claim 10, wherein the nitroxide radical-forming compound is an alkali metal nitrite or transition metal nitrite.

12. A method according to claim 11, wherein the nitroxide radical-forming compound is sodium nitrite.

13. A method according to any of claims 7 to 12, wherein the nitroxide radical-forming compound is a nitrovasodilator.

14. A method according to any of claims 7 to 13, wherein the reducing agent is cysteine, acetylcysteine, dithiothreitol, sodium dithionate, thiosalicyclic acid mercaptoethanol homocysteine, or ascorbate.

15. A method according to any of claims 7 to 14, wherein the nitroxide radical-forming compound is present in the mixture at a concentration of about 10 to 100 mM.

16. A method according to any of claims 7 to 15, wherein the amount of reducing agent is at least an equivalent of the radical-forming compound.

17. A method according to any of claims 7 to 16, wherein the mixture further includes a compound having superoxide dismutase activity.

18. A method according to claim 17, wherein the compound having superoxide dismutase activity is a copper complex.

19. A method according to claim 18, wherein the copper complex is superoxide dismutase, copper aspirinate, or indomethocin-copper.

20. A kit for preparing a unit dosage of a hair growth stimulating preparation, comprising:
a unit dosage amount of a pharmacologically acceptable nitroxide radical-forming compound in a pharmaceutical carrier;
a unit dosage amount of a pharmacologically acceptable reducing agent in a pharmaceutical carrier, the reducing agent being reactive with the radical-forming compound to form nitroxide radicals;
means for separating the unit dosage amounts from each other prior to dispensing; and
means for dispensing the unit dosage amounts.

21. A kit according to claim 20, wherein the unit dosage amounts are in a total volume of about 0.5 to 1.0 ml.

22. A kit according to claim 20 or 21, wherein the dispensing means includes means for mixing the unit dosage amounts.

23. A kit according to any of claims 20 to 22, wherein the separating means includes respective means for containing the unit dosage amounts.

24. A kit according to claim 23, wherein the containing means are sealed packets.

25. A kit according to any of claims 20 to 24, wherein the dispensing means includes a dual syringe.

26. A kit according to any of claims 20 to 25, wherein the radical-forming compound is an organic nitrate or an alkali metal nitrite.

27. A kit according to any of claims 20 to 26, wherein the unit dosage of the radical-forming compound further includes a compound having superoxide dismutase activity.

28. A kit according to any of claims 20 to 26, wherein the unit dosage of the radical-forming compound further includes an antioxidant.

29. A kit according to any of claims 20 to 26, wherein the unit dosage of the radical-forming compound includes a hydroxyl radical scavenger.

30. A composition for stimulating hair growth, comprising:
a pharmacologically acceptable stable free radical source compound and pharmacologically acceptable adjuvant selected from reducing agents, antioxidants and hydroxyl radical scavengers, in association with a topical pharmaceutical carrier.

31. A composition according to claim 30, wherein the free radical is selected from nitroxides and sulfoxides.

32. A composition according to claim 30 or 31, wherein the adjuvant includes a reducing agent.

33. A composition according to any of claims 30 to 32, wherein the free radical is nitroxide and the adjuvant includes a hydroxyl radical scavenger.

34. A composition according to any of claims 30 to 32, wherein the adjuvant includes an antioxidant.

35. A composition according to claim 34, wherein the antioxidant includes SODase activity.

36. A composition according to any of claims 30 to 35, further comprising an antiandrogen.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 30 0785

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X,P	EP-A-0 273 202 (E. VAN SCOTT, J.R.) * claims; examples 3;4,15 *	1,2,6,7,13-16,30-32	A 61 K 7/06 A 61 K 47/00
X,P	GB-A-2 198 132 (L'OREAL) * claims *	1,2,6,13,30,31,33,36	
X	WO-A-8 700 427 (PROCTOR P.H.) * claims *	1,2,6,13,30,31,33,36	
X	WO-A-8 600 616 (BAZZANO G.) * claims *	1,2,6,13,30,31,33,36	
X	WO-A-8 302 558 (BAZZANO G.) * claims *	1,2,6,13,30,31,33	TECHNICAL FIELDS SEARCHED (Int. Cl.4)
X	US-A-3 551 554 (R.J. HERSCHLER et al.) * column 32, lines 45-55; example 71; claims *	1,2,6,9,10,15,30,31,33	A 61 K
A		3-5,7,8,11-14,16-29,32	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	20-04-1989	SIATOU E

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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